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Toxicity Evaluation of Lower Fox River Water and Sediments

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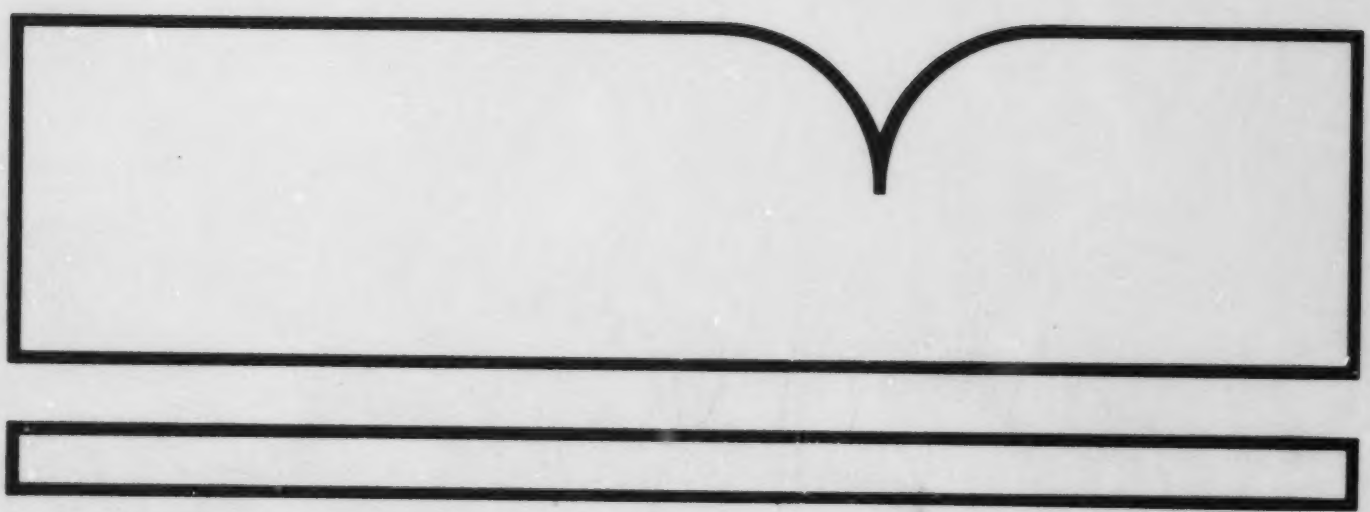

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Water and sediment were collected as grab samples from the Lower Fox River in late January, in mid-March, and in late April, 1985. Samples were transported to the Environmental Research Laboratory-Duluth (ERL-D) and a determination of their potential toxicity was accomplished through laboratory bioassays using four freshwater invertebrates and one freshwater vertebrate.

Results from the present toxicity evaluation of Lower Fox River water and sediment indicate a general absence of lethal effects as defined by the bioassays used and within the framework of the study. Significant sublethal effects were recorded in the form of reduced growth or fewer progeny, however, the effects were not observed for more than one species or testing period and no pattern was evident from this analysis.

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A Toxicity Evaluation of Lower Fox River Water and Sediments

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INTRODUCTION

The Lower Fox River in northeastern Wisconsin is approximately 64 km (40 miles) long and extends from the outlet of Lake Winnebago to Green Bay. This river is a major tributary to Green Bay, draining an area of 11,752 km^2 (~ 7% of the total drainage area of Lake Michigan). The fall of the Lower Fox River from its headwaters at Lake Winnebago to its mouth at Green Bay averages 1.3 meters per mile[1] and its flow is controlled by 11 dams, with the last dam occurring 11.7 km upstream from the mouth. The river is navigable through a series of locks.

The Lower Fox River is one of the most industrialized in the country receiving input from 14 pulp and/or paper mills, an electric generating facility and 8 municipal wastewater treatment plants serving approximately 250,000 people. Lake Winnebago, a hypereuthropic lake, is a major contributor of nutrients entering the river.

Until recently the Lower Fox River was one of the 10 most polluted rivers in the United States. Concerted efforts by the Fox Valley Water Quality Planning Agency and the Wisconsin Department of Natural Resources (WDNR) to clean up the river have reduced the organic wastes by 90%; the final cleanup to meet the standards set by Section 208 of the Clean Water Act is underway. Persistent, xenobiotic compounds continue to be a problem, however. A total of 105 organic compounds have been identified from biota, water, sediment, and effluent discharges[2]. Many of these chemicals are known to be toxic and/or bioaccumulate and are suspected of causing adverse effects to aquatic organisms. Toxics (via suspended solids, biota, and water) from the Lower Fox River enter Green Bay at Green Bay, Wisconsin. The zone of impact on the water quality of Green Bay extends as far at 15.5 km into the bay[3].

A previous study was conducted by personnel of the ERL-D to measure the total toxicity of industrial and municipal wastewater treatment effluents and their receiving waters from the Lower Fox River[4]. They could not attribute the toxicity they found to any one effluent. The present study was made to determine if there was instream toxicity and if bottom sediments from the Lower Fox River were toxic. Water and sediment samples were collected from several stations (Fig. 1) in the Lower Fox River and transported to ERL-D for testing.

Acute tests were performed using Daphnia magna on river aliquots and elutriate water. A 10 day D. magna chronic test was conducted on elutriate waters. A seven day Ceriodaphnia dubia life cycle test and a seven day fathead minnow (Pimephales promelas) subchronic test were conducted on river aliquots. Ten day tests for solid phase sediment toxicity were conducted using D. magna, Hyalella azteca and Ephemerella sp.

MATERIALS AND METHODS

Site Selection

Sampling sites on the Lower Fox River were selected in an effort to obtain an indication of instream toxicity, avoiding effluent plumes and immediate mixing zones as much as possible. Consideration was also given to the accessibility of the possible sites under both winter and spring conditions and the availability of sediment in the immediate area of the water sampling site. A station in Lake Winnebago (M) or a station in the river system upstream of any known point source discharges (L) were selected for "reference stations" for these experiments.

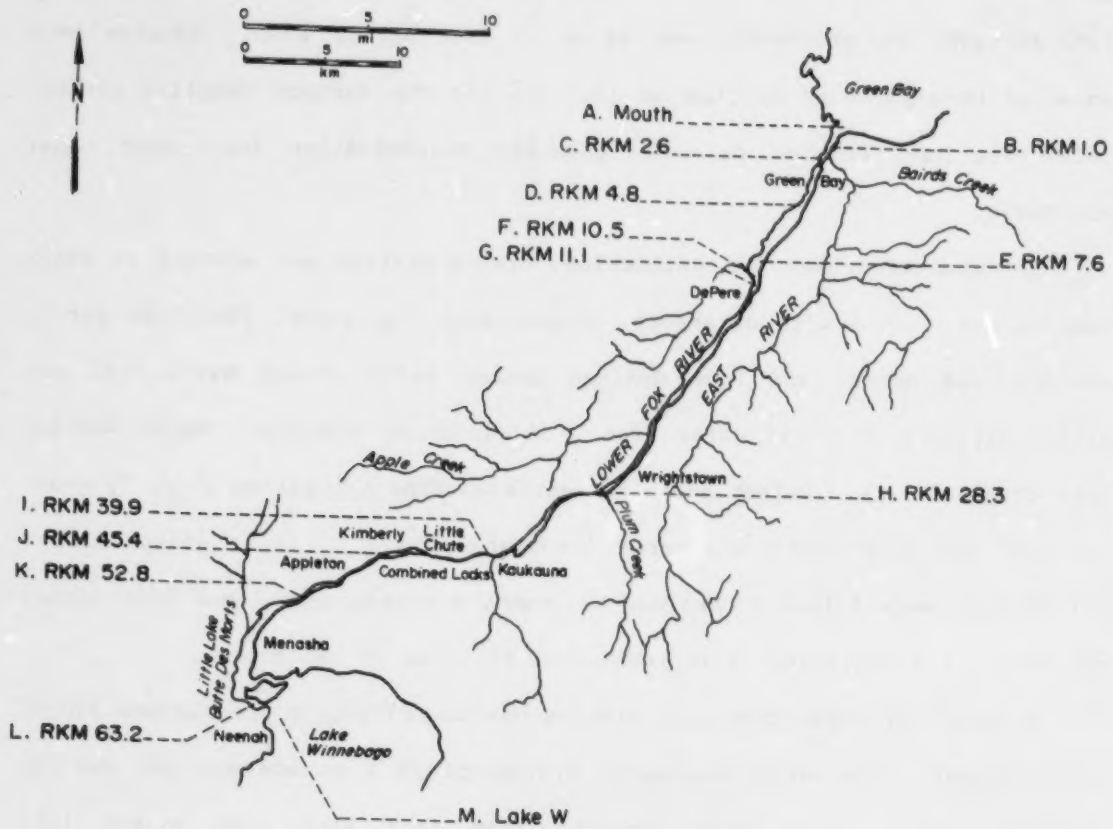

Figure 1. Map of Study Area and Locations of Sampling Stations for Toxicity Evaluation of Lower Fox River Water and Sediments (RKM = river kilometers).

Sampling Protocol

Water and sediment for bioassays and chemical analyses were collected as grab samples from the Lower Fox River, Wisconsin, and from Lake Winnebago in late January, in mid-March, and again in late April, 1985. Samples were obtained through holes drilled in the ice for the January sampling period. Holes were hand drilled to avoid possible contamination from power auger equipment.

All glassware used for collection, transportation and storage of water samples was washed with detergent, rinsed with tap water, distilled water, acetone, and hexane and then drained before being rinsed twice with one gallon aliquots of river water from the respective stations. Water samples were obtained from ~ 2 feet below the surface using a 1 gallon glass "jug-on-a-stick" and were then transferred immediately to a 19 liter glass bottle. All bottles were filled to exclude air and the covers were lined with aluminum foil. All containers were labeled at the time of collection.

A sample of water from each station was poured into a pre-cleaned 250 ml polyethylene bottle which contained 0.5 ml of 2N zinc acetate for sulfide analysis. Three liter water samples from each site were poured into calibrated, solvent rinsed glass bottles each of which contained 100 ml of (1:1) hexane/methylene chloride mixture. These samples were stored for possible future organic analysis.

Within 36 hours after collection all samples were transported to the laboratory and placed in a constant-temperature room which was maintained at 4° C. During transport from the field collection site to the laboratory, all samples were maintained at temperatures above freezing but below final test temperature.

Sediment samples were obtained from 11 locations (A-G, I-L) in the Lower Fox River and 1 station in Lake Winnebago (M) as grab samples using a standard size Ekman dredge. The nature of the bottom (i.e., hard, rocky) and accessibility precluded sampling for sediment at some of the sites. The depth of the river varied from 0.9 to 7.6 m. at the sites the sediments were obtained. Bottom material from each site was transferred from the dredge into pre-cleaned one gallon wide-mouth glass containers with foil lined covers. Sediment samples were then transported and stored in the same manner as the water samples.

Upon return to the laboratory the following sample splits were made: A) 250 ml subsamples of test water were poured into clean polyethylene bottles and acidified to 0.2% with HNO_3 in order to preserve the samples for metal analysis; B) 250 ml subsamples of test water were poured into clean polyethylene bottles and kept in the dark at 4° C for analysis of nitrate, nitrite, phosphate, chloride and sulfate. Upon completion of the anion analysis, the remainder of the sample was frozen and stored for future ammonia analysis; C) 250 ml subsamples of test water were poured into clean polyethylene bottles for pH, alkalinity, hardness and conductivity measurements.

Chemical Analysis

The pH was measured at 20° C according to EPA Method 150.1[5] using a Beckman model ϕ 70 pH meter standardized with pH 7.0 and 10.0 buffers before use. The conductivity was measured at 20° C according to EPA Method 120.1[5], using a YSI model 31 conductivity meter. Water hardness was measured according to EPA Method 130.2[5]. One hundred ml aliquots of samples were titrated with 0.01 M EDTA to a blue endpoint at pH 10 using Eriochrome Black T as an indicator. Alkalinity was measured according to EPA Method 310.1[5]. One hundred ml aliquots of samples were titrated with

0.02 N sulfuric acid to pH 4.4 endpoint using a Beckman model ϐ 70 pH meter which was standardized with pH 7.0 and 10.0 buffers before use. Dissolved oxygen was measured using a Beckman 0260 Oxygen Analyzer according to the manufacturer's instructions[6], Method 6.1.4.2, which specifies air-saturated water to calibrate the instrument.

Sulfides were analyzed according to EPA Method 376.1[5]. Iodine solution (0.025N) was added to 200 ml aliquots of water samples preserved with zinc acetate which were then titrated to a blue endpoint with 0.025 N PAO using starch as an indicator.

Anions (CL^-, NO_2^-, PO_4^{-3}, NO_3^-, SO_4^{-2}) were measured according to EPA Method 300.0[7] employing a model 12 Dionex (R) ion chromatography system with a Gilson (R) automatic sample changer. Five ml aliquots were placed in 12 X 75 mm plastic test tubes, capped with aluminum foil and placed in the automatic sample changer. All standard conditions were as specified in the EPA method with the conductivity detector set at the 300 umho range, pump volume at 2.5 ml/min. and sample loop volume at 1000 microliters.

Ammonia was measured on the ion chromatography system also; the manufacturers recommendations[8] for column types, eluent, and regenerant were followed. Operating conditions for ammonia analysis were as follows: Columns - 4 x 50 mm cation precolumn (Dionex P/N 030830), 4 X 250 mm cation separator column (Dionex P/N 030831), cation fiber suppressor (Dionex P/N 036179); detector - conductivity at 30 μmho scale; eluent - 0.003 N HCL; regenerant - 0.040 M TMAOH (Tetramethylammoniumhydroxide pentahydrate) at 3.1 ml/min.; sample loop - 500 microliters; pump volume - 2.5 ml/min.

At least one in ten samples were analyzed in duplicate for both anion and ammonia (cation) analysis as a check on the precision of the procedures.

Environmental Monitoring and Support Laboratory (EMSL) quality assurance (QA) samples were analyzed in each analytical run for all ions, except nitrite, as a check for the accuracy of the methods. There was no known QA sample available for nitrite at the time of analysis. A separate nitrite standard was analyzed in each analytical run as a check for any oxidation of nitrite to nitrate in the mixed standards. No oxidation was observed in this standard. At least one river sample for each analytical run was spiked with an anion or ammonia standard to insure that no matrix effects were present.

Bioassay Methods

A determination of the potential toxicity of water and sediments from the Lower Fox River was accomplished using four freshwater invertebrates and one freshwater vertebrate in the following laboratory bioassays: a Daphnia magna acute toxicity test; a Daphnia magna life cycle toxicity test; a subchronic fathead minnow (Pimephales promelas) toxicity test; a life cycle toxicity test using Ceriodaphnia; and a Hyalella and Ephemerella 10 day test. The species tested, stage of development, medium, type of test, measured response, duration, renewal, test solution volume, number of animals per replicate, number of replicates per treatment, food added, and the temperature for these tests are given in Table 1.

All animals used in the bioassays were obtained from ERL-D culture stock with the exception of the Ephemerella which were collected from the Sucker River in northeastern Minnesota. Test organisms were cultured at the same temperature as the test temperature with the exception of the Ephemerella, which were acclimated over a period of 7 days from an initial temperature of 4° C to the eventual test temperature of 20° C. The photoperiod and the feeding regimes were identical for both the culturing and the testing period.

Table 1. Summary of Test Conditions for Bioassay Experiments

	Daphnia magna	*Pimephales promelas*	*Ceriodaphnia dubia*	*Daphnia magna*	*Daphnia magna*	*Hyalella azteca*	*Ephemerella* sp.	*Daphnia magna*
Medium	river water	river water	river water	elutriate	elutriate	solid phase	solid phase	solid phase
Type of test	acute	subchronic	life cycle	acute	chronic	acute	acute	chronic
Stage of development	< 24 hrs old	< 24 hrs old	< 10 hrs old	5 or 6 day old	5 or 6 day old	mature	larvae	5 or 6 day old
Measured response	survival	survival growth	survival reproduction	survival	survival reproduction	survival	survival	survival reproduction
Duration	48 hrs	7 day	3 broods or 7 day	48 hrs	10 day	10 day	10 day	10 day
Renewal	static	daily	2-3 day	static	static	static	static	static
Test solution Volume (ml)	80	500	15	200	200	1000	1000	1000
# Animals/ replicate	5	10	1	5	5	5	5	10
# Replicates/ treatment	2 or 3	3	10	3	3	2 or 3	3	2 or 3
Food added	none	brine shrimp nauplii	YCTF	none	Selanastrum & trout food	Selanastrum & trout food	Selanastrum & trout food	Selanastrum & trout food
Test temp °C	20±1	25±1	25±1	20±1	20±1	20±1	20±1	20±1

Acute tests were chosen as an initial screening process. In addition, sub-chronic, partial life cycle, and life cycle tests were conducted to determine possible effects on survival, growth, or reproduction.

Test temperatures were maintained by partially submerging the chambers containing the water and/or sediment and the test organisms into thermostatically controlled water baths. A photoperiod of 16 hours was maintained for both the culture and exposure of test organisms. Water quality was routinely monitored to ensure it's adequacy for the test organisms. Test chambers for the D. magna acute tests, Ceriodaphnia life cycle tests and the elutriate bioassays were covered with glass to minimize evaporation.

All containers and other equipment coming in contact with test water and/or sediment used in these bioassays were constructed of glass or stainless steel. The cleaning protocol for all containers and equipment used in the laboratory for these tests included a detergent wash followed by three tap water rinses, three distilled water rinses, an acid (10% HNO_3) rinse followed by another three distilled water rinses, an acetone rinse, and finally by three distilled water rinses.

Individual bioassay procedures are described below for laboratory bioassays conducted with river water, liquid phase elutriate and solid phase.

A) Lower Fox River Water

Tests were conducted using Lower Fox River and Lake Winnebago water that had been stored at 4° C for not more than 7 days. Prior to being utilized for the bioassays described, a portion of the test water was rapidly warmed each day to test temperature and the water was then aerated briefly to stabilize dissolved gas concentrations.

1) Daphnia magna Acute Test

An evaluation of the acute toxicity of Lower Fox River water was conducted using the protocol established by EPA[9]. Culturing, handling and glassware cleaning procedures as outlined by EPA were followed with the exception of a dilution water rinse, due to the small amount of test water transported to and stored at the laboratory. The test solution was not renewed over the 48 hour period.

At the end of 24 and 48 hours the number of living daphnids were counted and recorded.

2) Fathead Minnow (Pimephales promelas) Sub-chronic Test.

The method described by Norberg and Mount[10] for measuring growth and survival of newly-hatched fathead minnow larvae was used for evaluating the subchronic effects of toxicants. Test chambers were modified to provide separate chambers for each of the replicates. All chambers were placed randomly in a temperature controlled bath. The test chambers measured 6 cm x 18 cm x 9 cm high and were filled to a volume of 0.5 liters. A stainless steel screen separated the chambers to form a 6 cm x 2.5 cm x 9 cm high sump on one end. This sump was useful for removing "old" test solution during the renewal process.

During the daily renewal process the animals were first counted and any dead fish were removed and recorded. Then waste products and dead brine shrimp nauplii were removed using a siphon tube similar to the one described by Norberg and Mount[10]. After the waste material and most of the "old" test solution was removed to a depth of 1 cm, the animals were again counted to assure that none had been accidentally removed by the siphoning before adding 0.5 liters of "new" test solution. Dissolved oxygen concentrations were measured periodically during the test, both on the "old" and the "new" test solutions.

The larval fish were fed 3 times per day at 5 hour intervals during the simulated daylight period with 0.05-0.1 ml of concentrated live brine shrimp nauplii (incubated 24 hours @ 28° C) that had been rinsed with distilled water.

At test termination, surviving fish were removed, counted, recorded, and dried to a constant weight (20-22 hrs @ 60° C). Final net dry weights for a composite of the surviving fish from each replicate chamber for each treatment were obtained utilizing an analytical balance (with an accuracy of .01 mg). Initial dry weights were obtained at the start of the test on four groups of ten fish (< 24 hrs old) by the same method.

3) Ceriodaphnia dubia Life Cycle Test

A life cycle test using Ceriodaphnia dubia and the method developed by Mount and Norberg[11] was conducted using Lower Fox River and Lake Winnebago waters. One animal < 10 hours old was placed into each of the 30 ml glass beakers filled with 15 ml of test water. This test water was warmed and aerated in the manner described above and dispensed using a 30 cc Manostat pipette.

The animals were fed 0.1 ml of YCTF (a suspension of yeast, a Cerophyl® extract, and a commercially formulated trout food) each day of the test.

The test solutions were renewed on day 3 and day 5 by filling cleaned, rinsed and labeled beakers with 15 ml of the "new" solution plus the food and then transferring the adult Ceriodaphnia from the "old" to the "new" solution with a pipette.

Survival of the original animals and the number of young produced were recorded on renewal days as well as on day seven. The number of young produced

in each brood was enumerated; differentiating broods by the relative size of the offspring. The measured responses were the number of original animals surviving beyond the third brood and the number of offspring produced in the first three broods.

B) Liquid Phase Elutriate

The techniques developed for evaluating toxicity of dredge samples[12] were employed in an effort to obtain an index of chemical toxicity for contaminants solubilized from the sediments into the water column. These tests may closely simulate the potential hazards encountered by aquatic organisms at the sediment-water interface. Daphnia magna was used as the test organism because it is very sensitive to industrial effluents and wastewater treatment discharges in the liquid phase[13].

The method employed in preparation of the liquid phase elutriate test solution consisted of: homogenizing the sediment grab sample by mechanical stirring; proportioning sediment to water at a 1:4 ratio (by volume); mechanical agitation of the sediment water combination for 1/2 hr; settling of the larger suspended solids for a minimum of 16 hrs; and finally, removal of a majority of all suspended solids by centrifuging for 45 min. to 1 hr at 2600 rpm. Reconstituted hard water[14] was used for the dilution water for the test conducted during the January 1985 period; river water from the respective stations was used for dilution water for the March and April testing periods. The leachate from this process was then decanted from centrifuge bottles and placed into test chambers; the solids were discarded.

1) Daphnia magna Acute Test

The Daphnia magna acute and chronic tests for the liquid phase elutriate experiments were conducted simultaneously in the same chamber (an acute test

was not conducted during the April testing period). At the start, 10 (5 or 6 day old) animals were placed into each beaker. No food was added to any of the containers during the acute phase of this test. Survivors were counted and recorded after 24 and 48 hours.

2) Daphnia magna Chronic Test

In conjunction with the above described acute test and after 48 hours had elapsed, the animals in each container were reduced to 5. Food consisting of 1 mg (oven dry weight) commercial trout food in a suspension and 2×10^7 cells Selanastrum was added to each beaker every 2-3 days after the second day. The chronic test ran for a total of 10 days after which time the number of surviving original test organisms and the number of offspring produced were counted and recorded.

C) Solid Phase

To allow the test organisms more direct access to all phases of chemicals in a system (solubilized, bound to suspended solids, and those incorporated in the sediments) a solid phase bioassay adapted from the method described by Nebeker et al.[15] was utilized.

Wet sediment was stored at 4° C for not more than 10 days before the solid phase bioassays began. From a homogenized sample of sediment from each station, 200 ml of sediment was subsampled and placed into each of 2 or 3 replicate 2 liter battery jars. Eight hundred ml of water from the respective station was then gently poured into each battery jar, bringing the total volume to 1,000 ml. These systems were left undisturbed overnight to allow the particulate matter to settle and to allow for exchange between the water and sediments. Before test organisms were introduced, aeration was provided for 1/2 hour through a glass Pasteur pipette with the tip submerged 2-3 cm below the surface of the water.

The test started when the animals were introduced and continued for a total of 10 days. Ten 5 or 6 day old Daphnia; 5 adult Hyalella; and for the March testing period, 5 larval Ephemerella were placed in each of 2 or 3 replicate chambers for each treatment. Stainless steel mesh bent into a U shape and measuring 6.5 cm x 6.5 cm was provided as a substrate for the Ephemerella. The test chambers were left uncovered and were aerated throughout the test. Deionized, distilled water was used to replace losses due to evaporation. Food was added to the systems every 2 to 3 days in the form of 5 mg (oven dry weight) commercial trout food in a suspension and 10^8 Selanastrum cells per chamber.

The test was terminated after 10 days by first counting and recording the number of surviving test organisms contained in the overlying water and then by screening the sediments (using a sieve with 500 micron openings) to recover the remainder. The number of original test animals surviving to test termination and the total number of daphnids produced in each chamber were recorded.

Statistical Analysis

All biological effects data were analyzed using a one-way analysis of variance and a two sided Dunnett's test ($p = 0.05$).

Quality Assurance

Sampling sites for this investigation were selected using the criteria outlined in the Description of Study Area section. The collection, identification, transportation, and storage of environmental samples were under the direct supervision of the first author. The first and second authors directly supervised all biological measurements and procedures and assigned

chemical samples to co-authors for analysis. Statistical analysis was conducted by the first author.

RESULTS

Physical and chemical measurements of the pH, alkalinity, conductivity, hardness, chloride, nitrite, nitrate, phosphate, sulfate, ammonia (total as NH_4^+ and unionized) and sulfide for all stations sampled on Lake Winnebago and the Lower Fox River are given in Table 2. The hydrogen ion activity was elevated in April compared to the sampling times in January or March by as much as a ten-fold increase. Alkalinity and hardness values were relatively constant over the sampling period. Conductivity measurements were consistently higher at station A (mouth of the river) than at stations upstream. Chloride concentrations were as great or greater at the mouth of the river when compared to stations upstream. Nitrite, phosphate and sulfide were below detection limits of the methods used. Nitrate concentrations were much higher at all Lower Fox River stations in March than in either January or April. For samples collected in April the sulfate concentrations and the total ammonia as NH_4^+ were considerably higher at station A than for other stations and times tested.

The values for the water quality parameters measured during the bioassays appeared adequate to meet the requirements of the test organisms.[9,10] The dissolved oxygen content of the water the fathead minnows (Pimephales promelas) were exposed to was $\geq$ 5.9 mg/1. Measurements of the dissolved oxygen content of the elutriate water revealed values $\geq$ 8.0 mg/1. The solid phase test systems were continually aerated. The pH ranged from 7.6-9.3, alkalinity from 134 to 172 mg/1 as $CaCO_3$, and hardness from 158 to 199 mg/1 as $CaCO_3$ for the Daphnia experiments.

Table 2. Physical and Chemical Data for Water Collected From the Lower Fox River at Various Locations and Dates

	Sampling Station[1]												
Jan. 30-31, 1985	A	B	C	D	E	F	G	H	I	J	K	L	M
pH	7.96	7.97	8.00	8.05	-	-	8.08	8.02	-	-	7.99	-	8.19
alkal mg/l	164.8	163.8	163.4	163.6	-	-	168.9	162.9	-	-	166.6	-	172.4
cond µs/cm	397	361	309	318	-	-	360	362	-	-	328	-	367
hardness mg/l	193.9	188.0	187.6	189.4	-	-	195.1	188.4	-	-	188.4	-	197.4
Cl mg/l	18	10	14	14	-	-	13	13	-	-	13	-	11
NO_2 mg/l	<0.2	<0.2	<0.2	<0.2	-	-	<0.2	<0.2	-	-	<0.2	-	<0.2
NO_3 mg/l	0.9	1.4	1.4	1.4	-	-	1.5	1.4	-	-	1.3	-	1.3
PO_4 mg/l	<0.2	<0.2	<0.2	<0.2	-	-	<0.2	<0.2	-	-	<0.2	-	<0.2
SO_4 mg/l	22.7	19.7	19.7	20.2	-	-	19.7	18.3	-	-	14.8	-	16.8
NH_4^+ mg/l (total)	0.56	0.24	0.26	0.23	-	-	0.23	0.16	-	-	0.05	-	0.34
NH_3-N mg/l[2]	0.015	0.007	0.008	0.008	-	-	0.008	0.005	-	-	0.002	-	0.015
sulfides mg/l	<1.0	<1.0	<1.0	<1.0	-	-	<1.0	<1.0	-	-	<1.0	-	<1.0
March 13-14, 1985													
temp °C[3]	3	3	3	3	-	-	3	-	3	3	3	-	-
pH	7.63	7.80	7.87	7.78	-	-	7.94	7.94	7.94	7.83	7.75	7.67	7.61
alkal mg/l	133.6	146.4	145.7	132.7	-	-	158.5	160.9	165.8	166.3	164.0	168.2	155.3
cond µs/cm	440	380	348	379	-	-	355	412	383	399	415	395	378
hardness mg/l	164.9	175.5	178.0	166.5	-	-	180.4	189.6	198.9	194.9	194.9	192.9	187.4
Cl mg/l	20	16	16	20	-	-	14	13	13	13	17	10	10
NO_2 mg/l	<0.05	<0.05	<0.05	<0.05	-	-	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
NO_3 mg/l	4.0	4.0	4.0	4.4	-	-	3.8	3.5	3.4	4.0	3.7	3.0	0.8
PO_4 mg/l	<0.15	<0.15	<0.15	<0.15	-	-	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
SO_4 mg/l	22.5	19.0	19.0	19.5	-	-	18.5	18.5	17.7	17.0	19.2	14.5	13.5
NH_4^+ mg/l (total)	1.07	0.40	0.44	0.64	-	-	0.24	0.18	0.13	0.16	0.28	0.10	0.05
NH_3-N mg/l[2]	0.014	0.008	0.010	0.012	-	-	0.006	0.005	0.003	0.003	0.005	0.002	0.001
sulfides mg/l	<1.0	<1.0	<1.0	<1.0	-	-	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
April 30, 1985													
temp °C[3]	18	17	17	17	18	19	19	-	18	18	18	17	-
pH	8.38	8.90	8.75	8.65	8.95	8.93	8.98	-	9.01	9.3	9.06	9.27	-
alkal mg/l	153.7	141.8	141.3	144.8	138.3	140.4	140.4	-	140.8	140.4	148.4	139.0	-
cond µs/cm	419	300	315	315	273	310	285	-	300	282	290	275	-
hardness mg/l	188.1	159.1	159.2	162.7	158.7	159.6	160.2	-	158.4	159.6	162.3	164.2	-
Cl mg/l	31.3	13.4	13.6	16.4	11.4	11.4	11.6	-	11.4	11.4	13.1	9.5	-
NO_2 mg/l	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	<0.05	<0.05	-
NO_3 mg/l	1.1	1.1	1.1	1.2	1.0	0.4	0.9	-	0.8	0.8	0.6	0.4	-
PO_4 mg/l	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	-	<0.10	<0.10	<0.10	<0.10	-
SO_4 mg/l	36.0	16.0	16.0	18.0	15.0	16.0	15.0	-	15.0	15.0	16.0	13.0	-
NH_4^+ mg/l (total)	1.93	0.26	0.24	0.33	0.28	0.40	0.26	-	0.30	0.33	0.28	0.16	-
NH_3-N mg/l[2]	0.102	0.048	0.035	0.039	0.057	0.078	0.055	-	0.066	0.115	0.069	0.051	-
sulfides mg/l	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	<1.0	<1.0	<1.0	<1.0	-

[1] See Figure 1

[2] Unionized ammonia calculated from total ammonia as NH_4^+ using the table in Appendix A-1 from Thurston et al. 1979[16].

[3] At time of collection

There was no significant adverse effect on survival of Daphnia, fathead minnow, or Ceriodaphnia exposed to Lower Fox River water over the entire testing period and for the various lengths of exposures (Tables 3, 4 and 5). There was, however, a significant adverse effect on growth of fathead minnows exposed to water from station D collected in January (Table 4), and water collected in March significantly reduced Ceriodaphnia reproduction for all test stations except L.

The elutriate water was not toxic to Daphnia in the acute tests (Table 6). Survival was $\geq$ 93% in the 10 day elutriate tests with the exception of station K in April when there was total mortality in 2 of 3 replicates. Elutriate water from station G in March and station K in April significantly reduced young production; however, Daphnia exposed to elutriate water from several stations had greater young production than those in reference waters.

Survival of Daphnia in the reference units for the January and March solid phase test was unsatisfactory (70% & 43%, respectively); however, survival was $\geq$ 80% for all other stations (Table 7). There was no significant adverse effect on survival of Daphnia in the solid phase test in April. Survival of Hyalella in the reference units of the solid phase test was $\geq$ 80% and there was no significant adverse effect on survival of Hyalella in the treatments over the entire testing period. Survival of Ephemerella exposed to the solid phase during the March testing period was less than satisfactory (73%) in the reference units and, therefore, no significance can be attributed to the response in the treatments. Reproduction of Daphnia was consistently lower in the reference units of the solid phase tests than in the treatments (Table 8).

Table 3. Mean Percent Survival of *Daphnia magna* Exposed to Lower Fox River Water For 48 hrs.

Stations	January	March	April
A	100	100	100
B	100	100	100
C	100	100	100
D	100	100	100
E	-	-	100
F	-	-	100
G	100	100	100
H	90	100	-
I	-	100	100
J	-	100	100
K	100	100	100
L	-	100	100+
M	100+	100+	-

+ Reference station

Table 4. Survival and Growth of Pimephales promelas Exposed to Lower Fox River Water For Seven Days.

Stations	Mean Percent Survival			Specific Growth Rate (%/Day)		
	January	March	April	January	March	April
A	97	93	90	22.9	28.7	29.7
B	83	93	97	23.0	29.9	30.7
C	80	87	100	25.8	29.5	30.6
D	90	98	87	20.7*	28.4	29.7
E	-	-	100	-	-	30.2
F	-	-	97	-	-	30.5
G	90	90	97	23.2	29.6	30.2
H	97	97	-	22.9	28.8	-
I	-	93	87	-	28.3	28.9
J	-	90	93	-	28.4	30.2
K	87	93	97	23.4	27.5	30.4
L	-	93	93+	-	29.6	29.7+
M	93+	97+	-	26.6+	29.2+	-

* Significantly less ($p = 0.05$) than reference station

+ Reference station

Table 5. Survival and Reproduction of Ceriodaphnia dubia Exposed to Lower Fox River Water.

Stations	Mean Percent Survival			No. of young in 3 broods per surviving female + SD		
	Jan	Mar	Apr	Jan	Mar	Apr
A	90	100	100	25 ± 5	22 ± 2*	29 ± 3
B	100	100	100	24 ± 4	21 ± 4*	25 ± 2
C	100	100	100	21 ± 7	20 ± 3*	25 ± 3
D	80	100	80	24 ± 4	21 ± 3*	26 ± 2
E	-	-	100	-	-	25 ± 3
F	-	-	100	-	-	27 ± 3
G	100	100	70	21 ± 9	21 ± 3*	27 ± 3
H	90	100	-	23 ± 5	22 ± 2*	-
I	-	100	100	-	23 ± 5*	25 ± 4
J	-	100	90	-	23 ± 3*	27 ± 2
K	90	100	80	22 ± 9	22 ± 2*	24 ± 4
L	-	100	90+	-	28 ± 3	26 ± 2+
M	100+	100+	-	22 ± 6+	28 ± 3+	-

* Significantly less ($p = 0.05$) than reference station

+ Reference station

Table 6. Survival and Reproduction of *Daphnia magna* in Elutriate Tests.

Stations	Survival in 48 hrs (mean percent)			Survival in 10 days (mean percent)			Young production/chamber (mean + S.D.)		
	Jan	Mar	Apr	Jan	Mar	Apr	Jan	Mar	Apr
A	100	100	-	100	100	100	119 ± 13	43 ± 6	93 ± 6
B	100	100	-	93	100	100	94 ± 12	34 ± 5	78 ± 8
C	100	100	-	100	100	100	95 ± 14	29 ± 10	75 ± 5
D	100	100	-	100	100	100	125 ± 16	49 ± 6	95 ± 18
E	-	-	-	-	-	100	-	-	72 ± 5
F	-	-	-	-	-	100	-	-	68 ± 3
G	100	100	-	100	100	100	101 ± 8	21 ± 3*	81 ± 8
H	-	-	-	-	-	-	-	-	-
I	-	100	-	-	100	100	-	29 ± 3	55 ± 14
J	-	100	-	-	100	100	-	24 ± 4	89 ± 7
K	-	100	-	-	100	33*	-	24 ± 4	22 ± 38*
L	-	100+	-	-	100+	100+	-	35 ± 5+	74 ± 7+
M	100+	-	-	100+	-	-	73 ± 23+	-	-

* Significantly less ($p = 0.05$) than reference station

+ Reference station

Table 7. Mean Percent Survival of *Daphnia magna*, *Hyalella azteca* and *Ephemerella sp.* in Solid Phase Tests.

Stations	Daphnia			Hyalella			Ephemerella
	Jan	Mar	Apr	Jan	Mar	Apr	Mar
A	93	97	100	67	80	90	47
B	97	83	100	87	87	100	60
C	100	100	95	100	93	90	40
D	97	100	100	80	100	90	67
E	-	-	100	-	-	80	-
F	-	-	60	-	-	50	-
G	97	87	100	87	93	80	73
H	-	-	-	-	-	-	-
I	-	100	90	-	67	100	53
J	-	100	100	-	73	80	87
K	-	90	100	-	87	100	67
L	-	43+	95+	-	80+	90+	73+
M	70+	-	-	90+	-	-	-

+ Reference station

Table 8. Reproduction of Daphnia magna in Solid Phase Tests.

Station	Daphnia magna reproduction (number of young/chamber + S.D.)		
	January	March	April
A	335 ± 49	371 ± 91	393 ± 52
B	313 ± 30	325 ± 73	421 ± 91
C	326 ± 30	312 ± 25	418 ± 14
D	315 ± 5	332 ± 40	291 ± 10
E	-	-	327 ± 33
F	-	-	270 ± 147
G	317 ± 21	280 ± 80	405 ± 105
H	-	-	-
I	-	377 ± 35	382 ± 212
J	-	226 ± 63	223 ± 27
K	-	369 ± 53	482 ± 146
L	-	93 ± 60+	268 ± 1+
M	197 ± 50+	-	-

+ Reference station

SUMMARY

Physical and inorganic chemical measurements made on Lower Fox River water show few abnormal values. Unionized ammonia (NH_3-N) levels were higher in April due to the influence of an increase in pH during that period on the relative percentage of unionized to total ammonia. Nitrate concentrations were elevated in March compared to the January or April study periods. Conductivity, ammonia, chloride, and sulfate values all measured higher at station A compared to the other stations. Dissolved oxygen measurements obtained from the WDNR[17] for the Lower Fox River range from 12.0 - 17.9 mg/l for January, 12.7 - 18.0 mg/l for February, 9.9 - 17.4 mg/l for March, and 7.5 - 17.9 mg/l for April, 1985.

Results from the present study of Lower Fox River water indicate a general absence of lethal effects as defined by the bioassays used and within the temporal and spatial framework of the study. Significant sublethal effects resulting from exposure to Lower Fox River water included reduced growth of fathead minnows for station D in January and fewer Ceriodaphnia progeny for stations A-K in March.

The liquid phase elutriate test showed few lethal effects with the exception of total mortality of Daphnia magna in two of three replicates for station K in April; the third had 100% survival. Production of young Daphnia varied greatly in the elutriate test with two stations (G in March and K in April) producing significantly fewer young than the reference station (L) and three stations (A and D in January and D in March) producing significantly more young than the reference station.

Survival and reproduction of Daphnia magna in the solid phase test was often less for the reference stations than for the other stations. The solid

phase was not toxic to Hyalella in the 10 day tests. Ephemerella were not well suited for the static environment of the solid phase test.

DISCUSSION

Laboratory bioassays using Lower Fox River water and sediment failed to reveal consistent lethal effects. A pathogen may have been the cause for the total mortality of Daphnia observed in two of three replicates for Station K in the elutriate tests in April. The observed presence of indigenous zooplankton (Crustacea) in water collected for these experiments further substantiates the lack of mortality recorded in the bioassays. Consequently, an analysis of contaminants in Lower Fox River water was not undertaken.

No pattern was evident for the sublethal effects observed and the effects were not observed for more than one species or testing period. The cause-effect relationship for the significantly fewer Ceriodaphnia produced in March is not known. In the elutriate tests, nutrients present in the water and those released from the sediment may have been more of a causative agent for the variability observed in young production than a toxic influence.

Cairns et al.[18] reported acute toxicity to Daphnia magna exposed to liquid phase elutriate from two stations on the Lower Fox River (mouth and 0.8 km upstream) collected in 1982. Their measurements of suspected toxicants showed all were below the acutely toxic level. A second testing yielded no significant mortality.

A previous investigation by personnel of ERL-D[4] also indicated no toxicity to fathead minnows (Pimephales promelas) or Ceriodaphnia in ambient tests conducted in 1983. The investigators did, however, record adverse

effects with dilution or receiving water used in conjunction with effluent toxicity testing. In a personal communication, D. McCauley[19] reported a lethal effect on fathead minnows (Pimephales promelas) under test conditions similar to the ones described herein with water collected near the DePere Dam (in the vicinity of Station G) in April, 1984. Toxicity events in the Lower Fox River appear to be episodic in nature.

Several limitations of the present investigation's ability to accurately assess potential instream toxicity are evident. The limitations of a grab sample at particular points in time are inherent. The process(es) occurring during prolonged storage and the resulting effects on the integrity of water samples are not well defined. It is likely that aeration used to stabilize dissolved gases for the bioassays may have altered the environmental samples and their potential toxicity. Also, the influence of laboratory bioassay temperature conditions on potential toxicity is open to discussion. In situ bioassays and instream measurements could more accurately evaluate potential instream toxicity in an environment such as the Lower Fox River.

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